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Phytochemical screening and antimicrobial activity of methanol extract of five medicinal plants in Lau Local Government Area of Taraba State, Nigeria

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ABSTRACT

The present study was carried out to determine the phytochemical investigation and antimicrobial activity of methanol extract of five medicinal plants in Lau Local Government area of Taraba state, these medicinal plants include *Eucalyptus citriodora, piliostigma thonnigii, Acacia nilotica wild, Cassia italica and Senna siamea.* Maceration method of extraction was used. The results of the phytochemicals screening of the five medicinal plants extract revealed the availability of alkaloid, flavonoid, saponin, steroids, tannin and cardiac glycosides in *acacia nilotica wild* while in other plants one or two phytochemicals were not detected. The antimicrobial activity was also carried out on both the five methanolic extracts against four bacteria, *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Esherichia coli and Salmonella typhi,* Gentamycin was used as control standard. The concentrations prepared ranged from 12.5, 25, 50 and 100 mg/mL of the plants extract against 5 mg/mL of Gentamycin. The results of the susceptibility test showed significant ($p \leqslant 0.05$) inhibitory effects of the extracts against all the test organisms with mean zones of inhibition range of 16±0.00 - 6±0.00, 16±0.61 -6±0.00, 19±0.35-9±0.00 and 11±0.00-6±0.00 for *piliostigma thonnigii*, 22±075-15±0.35, 23±0.35-16.0.25, 24±0.00-17±0.06 and 21±031-0±0.00 for *Acacia nilotica wild,* 18±0.30-10±0.30, 17±0.00-11±0.30, 19±0.72-9±0.00 and 18±1.30-11±0.00 for *Eucalyptus citriodora,* 21±0.26-9±0.00, 19±0.00-9±0.00, 17±0.75-9±0.25 and 17±0.20-9±0.00 for *Cassia italica* and 18±0.00-10±0.00, 17±0.32-11±0.00, 19±0.06-9±0.00 and 18±0.00-11±0.35 for *Senna siamea*. The MIC and MBC against the test organisms were 12.5mg/ml and 25mg/ml for *Staphylococus aurous,* 6.25mg/ml and 12.5mg/ml for *Pseudomonas aeruginosa*, 3.12mg/ml and 6.25mg/ml for *Esherichia Coli* and 1.56mg/ml and 3.12mg/ml for *Salmonella typhi.* these results justify the uses of these medicinal plants for the treatment of different bacterial infections.

Keywords: Antimicrobial activity, Bacterial isolates, Investigations, Medicinal plants, Phytochemicals

Introduction

The attempt by mankind to use plant products to cure diseases and relieve physical suffering is as old as creation. Humans have derived many uses and benefits from the plants indigenous to their locality and have resorted to nature, mainly plants for medicine and health sources [1]. The indigenous medicinal plants form an important component of the natural wealth and culture of a geographical area. The study of plant began with tribal efforts to identify edible, medicinal and poisonous plants making botany one of the oldest sciences, of all the species of plants studied only about 60 have been evaluated Phyto-chemically [2]. Plants have been used as drugs for centuries, initially as traditional preparations [3]. The use of plant species as traditional medicines provides a real substitute to orthodox healthcare services for rural communities of the developing nations [4]. It has been estimated that around 80% of the population in developing countries depends on traditional medicines for primary health care system. These traditional medicines are cost-effective, safe and affordable [5].

Globally, approximately 85% of the traditional medicines used in primary healthcare are derived from plant species [6]. Therefore, medicinal plants are the indigenous heritage of global importance [7]. The utilization of plants and their resources for combating various ailments predates written history and they are still in use all over the world. The oldest written evidence of the usage of medicinal plants for the preparation of drugs has been found on a Sumerian clay slab from Nagpur, which is believed to be approximately 5000 years old.

This study aimed at phytochemicals and antimicrobial screenings of five medicinal plants in Lau Local Government Area, Taraba state, Nigeria. The specific objectives of this study are to extract the five plants; *Eucalyptus citriodora, piliostigma thonnigii, Acacia nilotica wild, Cassia italica and Senna siamea.* carryout phytochemical screening and antimicrobial activity on the five plants extracts.

Material and Methods

Equipment/Apparatus

Laboratory ware, pestle and mortar, analytical balance, sieve and filter paper. Acetic acid, Chloroform, Tetraoxosulphate (VI) acid (H_2SO_4, SG. 1.8496%), Distilled water, Trioxonitrate (V) acid (HNO_3, S.G 1.42, 72%), Hydrochloric acid (HCl, SG. 1.18.36%), potassium hydroxide, Methanol (96%v/v), Fehling's Solution, Sodium hydroxide etc. All reagents were of analytical grade.

Solvent used for extraction

Methanol (96%v/v).

Plants collection and Authentication

Sampling was done according to the method [8]. Fresh plants of five different medicinal (*Eucalyptus citriodora, piliostigma thonnigii, Acacia nilotica wild, Cassia italica and Senna siamea.*) was collected and authenticated at the department of biological science (botany), Taraba State University, Jalingo. The plants were then collected at random from different location in Lau local government Area of Taraba state and transported to the Chemistry Laboratory, Taraba University State, Jalingo where they were prepared for the analysis. All the plant parts were allowed to dry under shade on the day of collection to preserve as much as possible physiological changes taking place before extraction [9].

Plant Extraction

The medicinal plants were washed, air dried and later oven dried at room temperature (26 °C) for seven days. The dried leaves, stem or bark was pounded into a homogenous powder using mortar and pestle. The plant materials were then stored in specimen bottles for future use. Methanol was used for extraction. 10geach of the dry plant materials was soaked in 120mL of methanol at room temperature for two days. The extracts were filtered using a Whatmann filter paper No. 42 (125 mm), and then through cotton wool. The extracts were evaporated into dryness using a hot water bath for 72 hours, and was then stored in the refrigerator for further use.[10].

Standardization of inoculum

Fresh cultures (18-24hours) of the bacterial isolates were used to prepare the inoculum. The organisms were suspended in sterile normal saline, and the turbidity was adjusted to match the 0.5 McFarland standard. Bacterial suspensions were prepared from pure cultures grown on nutrient agar and standardized to approximately $1.0X10^8$ CFU/ml. [11].

Preparation of different concentration of the plants extract

A stock solution was prepared by dissolving 0.5 g of each of the five plants extracts in 5 ml of sterile distilled water. Serial two-fold dilutions were then carried out in four test tubes containing 2 ml of distilled water each to obtain concentrations of 100, 50, 25, and 12.5 mg/ml. Gentamycin (5 mg/ml) served as the positive control for the test isolates. [12].

Test organisms

The test organisms or bacterial isolates used in these studies are *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Esherichia coli and Salmonella typhi.*

Antimicrobial Susceptibility test

Before every antimicrobial test, the stock was kept on nutrient agar plates and cultivated for incubation at 37 °C. The discs were sterilized in an oven at 121 °C for 15 minutes after being prepared with Whatman filter paper No. 42 (125 mm) and stored in vitals bottles. Using a sterilizer for the caps in each instance, prepared discs containing the different extracts were carefully placed on the inoculation plates [13]. Then, the plates were inverted and inoculated for 24 hours at 37 °C in an incubator. The test organisms' growth and inhibition—both sensitive and non-sensitive were taken into account while determining the results

Statistical Analysis

Each experiment of the antimicrobial activities was carried out in triplicates and the results obtained was subjected to Analyses of Variance (ANOVA) and expressed as mean ± Standard Deviation.

Results and Discussion

Table 1. Phytochemical screening test for leaves, roots and stem extracts of five different medicinal plants (*Eucalyptus citriodora, piliostigma thonnigii, Acacia nilotica wild, Cassia italica and Senna siamea*)

Phytochemical	*Eucalyptus citriodora*	*Piliostigma thonnigii*	*Acacia nilotica willd*	*Cassia italica*	*Senna siamea*
Alkaloid	+	+	+	-	+
Flavonoid	-	+	+	+	+
Saponin	+	+	+	+	+
Steroids	-	-	+	+	+
Tannin	+	+	+	+	-
Cardiac glycosides	-	+	+	-	+

Key: + = detected, - = not

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Table 1 shows the results of a phytochemical screening test for five different medicinal plants. The presence (+) or absence (-) of certain phytochemicals in the leaves, roots, and stem extracts of these plants is indicated. From the results, *Acacia nilotica* plant shows the presence of all the tested phytochemicals and in the other plants, some of the phytochemicals was not detected [14].

Table 2. Antimicrobial activity of methanolic extract of stem bark of *P. thoningii* plant (Zone of inhibition in mm)

Solvent	Conc (mg/mL)	Organisms *S.a*	*P.s*	*E.Coli*	*S.t*
Methanol extract	100	16 ± 0.00	16 ± 0.61	19 ± 0.35	11± 0.00
	50	14 ± 0.35	15 ± 0.00	14.2± 0.35	9± 0.00
	25	11 ± 0.00	10 ± 0.00	11 ± 0.06	8± 0.00
	12.5	6 ± 0.00	6 ± 0.00	9± 0.00	6± 0.00
Controls (GTM)	**5** ± 0.00	22± 0.00	20± 0.00	17± 0.00	21± 0.00

Values are presented as Mean ± standard deviation, p≤0.05
Key: S.a = *Staphylococus aureus*; P.s = *Pseudomonas aeruginosa*; E.Coli = *Esherichia Coli and Salmonella typhi*; GTM = Gentamycin

Table 2 shows the stem bark methanolic crude extract of *P. thoningii* plant's antibacterial activity. *E. coli*, shows the maximum and lowest zones of inhibition of 19±0.35 mm and 9±0.00mm, respectively. *S. aureus*. Shows the maximum and lowest zones of inhibition of 16±0.00 mm and 6±0.00mm, respectively and *Pseudomonas,* had zones of inhibition 16±0.61mm and 6±0.00 mm, respectively. Similarly, *Salmonella typhii*, had the lowest zone of inhibition was 6±0.00 mm. The results obtained, showed strong antimicrobial activity, which may be due to the bioactive constituents, such as alkaloids, tannins, and flavonoids, present in the extracts 15.

Table 3. Antimicrobial activity of methanolic extract of *Acacia nilotica* plant (Zone of inhibition in mm)

Solvent	Conc. (mg/mL)	Organisms *S.a*	*P.s*	*E.Coli*	*S.t*
Methanol extract	100	22 ± 0.75	23 ± 0.35	24 ± 0.00	21 ± 0.31
	50	19 ± 0.35	21 ± 1.05	22 ± 0.25	17 ± 0.00
	25	18 ± 0.00	18 ± 0.00	20 ± 0.71	1 5± 0.00
	12.5	15 ± 0.35	16 ± 0.25	17 ± 0.06	0 ± 0.00
Controls (GTM)	**5** ± 0.00	25 ± 0.00	24 ± 0.00	23 ± 0.00	21 ± 0.00

Values are presented as Mean ± standard deviation, p≤0.05
Key: S.a = *Staphylococus aurous*; P.s = *Pseudomonas aeruginosa*; *E. Coli* = *Esherichia Coli* and *Salmonella typhi*; GTM = Gentamycin

Table 3 shows the antimicrobial activity of the methanolic extract of the *Acacia nilotica* plant against various organisms, measured by the zone of inhibition in millimeters (mm). The organisms tested include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. Coli*, and *Salmonella typhi*. *Staphylococcus aureus* had zone of inhibition ranges from 22±0.75 mm at a concentration of 100 mg/mL to 15±0.35 mm at a concentration of 12.5 mg/mL. This indicates that the extract has a significant inhibitory effect on *Staphylococcus aureus*, and the effect decreases as the concentration of the extract decreases. *Pseudomonas aeruginosa* had zone of inhibition ranges from 23±0.3 mm at a concentration of 100 mg/mL to 16±0.25 mm at a concentration of 12.5 mg/mL. Similar to *Staphylococcus aureus*, the extract shows a strong inhibitory effect on P.s, which also decreases as the concentration decreases. *E. coli* had the highest zone of inhibition, ranging from 24±0.00 mm at a concentration of 100 mg/mL to 17±0.06 mm at a concentration of 12.5 mg/mL. This suggests that the extract is most effective against *E. coli* among the tested organisms [16]. Salmonella typhi had zone of inhibition ranges from 21±0.31mm at a concentration of 100 mg/mL to 0.00±0.00mm at a concentration of 12.5 mg/mL. This suggests that the extract also has a significant inhibitory effect on *Salmonella typhi*, but the effect at a concentration of 12.5 mg/mL is not known [17]. These results suggest that the methanolic extract of the *Acacia nilotica* plant has potent antimicrobial properties against the tested organisms.

Table 4. Antimicrobial activity of methanolic extract of *Eucalyptus citriodora* plant (Zone of inhibition in mm)

Solvent	Conc. (mg/mL)	Organisms *S.a*	*P.s*	*E.Coli*	*S.t*
Methanol extract	100	18 ± 0.30	17 ± 0.00	19 ± 0.72	18 ± 1.30
	50	15 ± 0.25	15 ± 0.35	14 ± 0.00	16 ± 0.25
	25	14 ± 0.00	13 ± 0.00	10 ± 0.25	13 ± 0.00
	12.5	10 ± 0.30	11 ± 0.30	9 ± 0.00	11 ± 0.00
Controls (GTM)	**5** ± 0.00	23 ± 0.00	18 ± 0.00	21 ± 0.00	20 ± 0.00

Values are presented as Mean ± standard deviation, p≤0.05
Key: S.a = *Staphylococus aurous*; P.s = *Pseudomonas aeruginosa*; E. Coli = *Esherichia Coli* and *Salmonella typhi;* GTM = Gentamycin

Table 4. shows the antimicrobial activity of the methanolic extract of the *Eucalyptus citriodora* plant against various organisms, measured by the zone of inhibition in millimeters (mm). The organisms tested include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. Coli,* and *Salmonella typhi*. *Staphylococcus aureus* shows zone of inhibition ranges from 18±0.30 mm at a concentration of 100 mg/mL to 10 ±0.30mm at a concentration of 12.5 mg/mL. This indicates that the extract has a significant inhibitory effect on *Staphylococcus aureus*, and the effect decreases as the concentration of the extract decreases. [18; 19]. *Pseudomonas aeruginosa* had zone of inhibition ranges from 17 ±0.00mm at a concentration of 100 mg/mL to 11± 0.30mm at a concentration of 12.5 mg/mL. Similar to *Staphylococcus aureus*, the extract shows a strong inhibitory effect on *Pseudomonas aeruginosa*, which also decreases as the concentration decreases. *E. coli* had the highest zone of inhibition ranging from 19±0.72 mm at a concentration of 100 mg/mL to 9± 0.00mm at a concentration of 12.5 mg/mL. This suggests that the extract is most effective against *E. Coli* among the tested organisms. *Salmonella typhi* had zone of inhibition ranges from 18±1.30 mm at a concentration of 100 mg/mL to 11±0.00mm at a concentration of 12.5 mg/mL. This suggests that the extract also has a significant inhibitory effect on *Salmonella typhi.* These results suggest that the methanolic extract of the *Eucalyptus citriodora* plant has potent antimicrobial properties against the tested organisms [20].

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Table 5. Antimicrobial activity of methanolic extract of *Cassia italica* plant (Zone of inhibition in mm)

Solvent	Conc. (mg/mL)	Organisms *S.a*	*P.s*	*E.Coli*	*S.t*
Methanol extract	100	21± 0.26	19± 0.00	17± 0.75	17± 0.20
	50	15± 0.00	14± 0.00	15± 0.00	16± 0.00
	25	10± 0.00	11± 0.00	10± 0.00	11± 0.08
	12.5	9± 0.00	9± 0.00	9± 0.25	9± 0.00
Controls (GTM)	**5** ± 0.00	29± 0.00	25± 0.00	23± 0.00	20± 0.00

Values are presented as Mean ± standard deviation, p≤0.05
Key: S.a = *Staphylococus aurous*; P.s = *Pseudomonas aeruginosa*; E.Coli = *Esherichia Coli and Salmonella typhi*; GTM = Gentamycin

The results from Table 5. shows antimicrobial activity of the methanolic extract of the *Cassia italica* plant against various organisms, measured by the zone of inhibition in millimeters (mm). The organisms tested include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. Coli*, and *Salmonella typhi*. At a concentration of 100 mg/mL, the zones of inhibition for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, E. coli, and *Salmonella typhi* were 21±0.26 mm, 19±0.00mm, 17±0.75mm, and 17±0.20 mm, respectively. As the concentration decreases, so does the zone of inhibition, indicating a dose-dependent antimicrobial activity. *Staphylococcus aureus* shows zone of inhibition ranges from 21±0.26 mm at a concentration of 100 mg/mL to 9±0.00mm at a concentration of 12.5 mg/mL. This indicates that the extract has a significant inhibitory effect on *Staphylococcus aureus*, and the effect decreases as the concentration of the extract decreases [21].

Similarly, *Pseudomonas aeruginosa* had zone of inhibition ranges from 19±0.00mm at a concentration of 100 mg/mL to 9±0.00mm at a concentration of 12.5 mg/mL. Similar to *Staphylococcus aureus*, the extract shows a strong inhibitory effect on *Pseudomonas aeruginosa*, which also decreases as the concentration decreases. *E. Coli* had zone of inhibition ranges from 17 ±0.75mm at a concentration of 100 mg/mL to 9±0.25 mm at a concentration of 12.5 mg/mL. This suggests that the extract is effective against *E. Coli* among the tested organisms [22]. *Salmonella typh*i also had zone of inhibition ranges from 17±0.20mm at a concentration of 100 mg/mL to 9±0.00mm at a concentration of 12.5 mg/mL. This suggests that the extract also has a significant inhibitory effect on *Salmonella typh*i. These results suggest that the methanolic extract of the *Cassia italica* plant has potent antimicrobial properties against the tested organisms [23].

Table 6. Antimicrobial activity of methanolic extract of *Senna siamea* plant (Zone of inhibition in mm)

Solvent	Conc (mg/mL)	Organisms *S.a*	*P.s*	*E.Coli*	*S.t*
Methanol extract	100	18 ± 0.00	17 ± 0.32	19 ± 0.06	18 ± 0.00
	50	15 ± 0.00	15 ± 0.00	14 ± 0.00	16 ± 0.00
	25	14 ± 0.00	13 ± 0.00	10 ± 0.00	13 ± 0.00
	12.5	10 ± 0.00	11 ± 0.28	9 ± 0.00	11 ± 0.35
Controls (GTM)	**5** ± 0.00	20 ± 0.00	18 ± 0.00	23 ± 0.00	22 ± 0.00

Values are presented as Mean ± standard deviation, p≤0.05
Key: S.a = *Staphylococus aurous*; P.s = *Pseudomonas aeruginosa*; E.Coli = *Esherichia Coli and Salmonella typhi*; GTM = Gentamycin

Table 6 shows the antimicrobial activity of the methanolic extract of the *Senna siamea* plant against various organisms, measured by the zone of inhibition in millimeters (mm). The organisms tested include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli,* and *Salmonella typhi*. *Staphylococcus aureus* exhibit zone of inhibition ranges from 18±0.00mm at a concentration of 100 mg/mL to 10±0.00mm at a concentration of 12.5 mg/mL. This indicates that the extract has a significant inhibitory effect on *Staphylococcus aureus* and the effect decreases as the concentration of the extract decreases [24]. *Pseudomonas aeruginosa* had zone of inhibition ranges from 17±0.32 mm at a concentration of 100 mg/mL to 11±0.28mm at a concentration of 12.5 mg/mL. Similar to *Staphylococcus aureus*, the extract shows a strong inhibitory effect on *Pseudomonas aeruginosa*, which also decreases as the concentration decreases. *E. coli* showed highest zone of inhibition, ranging from 19±0.06mm at a concentration of 100 mg/mL to 9±0.00mm at a concentration of 12.5 mg/mL. This suggests that the extract is most effective against *E. coli* among the tested organisms. *Salmonella typhi* also shows zone of inhibition ranges from 18±0.00mm at a concentration of 100 mg/mL to 11±0.35mm at a concentration of 12.5 mg/mL. This suggests that the extract also has a significant inhibitory effect on *Salmonella typhi*. These results suggest that the methanolic extract of the *Senna siamea* plant has potent antimicrobial properties against the tested organisms [25].

Table 7. Minimum inhibitory concentration (mic) and minimum bactericidal concentration

Organisms	MIC (mg/mL)	MBC (mg/mL)
Staphylococus aurous	12.5	25
Pseudomonas aeruginosa	6.25	12.5
Esherichia Coli	3.12	6.25
Salmonella typhi	1.56	3.12

Key: MIC= Minimum Inhibitory Concentration.
MBC= Minimum Bactericidal Concentration

Table 7 above, show the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of methanol extract of *Acacia nilotica* willd against the test organisms. The MIC and MBC against the test organisms were 12.5mg/ml and 25mg/ml for *Staphylococus aurous,* 6.25mg/ml and 12.5mg/ml for *Pseudomonas aeruginosa*, 3.12mg/ml and 6.25mg/ml for *Esherichia Coli* and 1.56mg/ml and 3.12mg/ml for *Salmonella typhi*

Conclusions

The findings highlight the rich phytochemical composition of the five medicinal plants (*Eucalyptus citriodora, piliostigma thonnigii, Acacia nilotica wild, Cassia italica and Senna siamea)* and underscore their potential therapeutic value. However, *Acacia nilotica wild* showed the broadest phytochemicals profile and highest antimicrobial activity, warranting further isolation of active compounds while others showed less phytochemical profile.

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More research is needed to fully understand the extent of these active compounds present in *Acacia nilotica* wild and how they can be best utilized in human health. The results suggest a dynamic interplay between traditional and modern health-care practices, with potential implications for the conservation of medicinal plants and the preservation of traditional knowledge. The significant antimicrobial activity observed against various pathogens suggests that *Acacia nilotica* wild could be further explored for the development of new antimicrobial drugs. However, further studies are needed to identify the specific compounds responsible for the observed antimicrobial activity and to evaluate their safety and efficacy in vivo.

Author Contributions

TR: Conceptualization, Writing Original draft and Funding Acquisition. ICA: Investigations, data curation and Formal Analysis. LA: Supervision, Funding Acquisition and Writing Review and Editing. FU: Methodology, Formal Analysis, Writing Review and Editing. IM: Writing Original draft, data curation and Funding Acquisition. All authors then critically reviewed the following versions. All authors have read and approved the final version of the paper and its submission and have given consent for publication.

Declaration of Generative AI and AI-Assisted Technologies in Scientific Writing

Nothing to disclose.

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Data Availability Statement

All relevant data are available within the manuscript

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None

Conflict of Interest

The authors declare that they have no conflicts of interest.

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